Effect of Bevacizumab on Inflammation and Proliferation in Human Choroidal Neovascularization

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Objective: To evaluate the effect of bevacizumab (Avastin; Genentech, Inc, South San Francisco, California) on inflammation and proliferation in human choroidal neovascularization (CNV) secondary to age-related macular degeneration.

Methods: Retrospective review of interventional series of 38 patients who underwent choroidal neovascular membrane (CNVM) extraction. Twenty-four patients received intravitreal bevacizumab 1 to 154 days preoperatively (bevacizumab CNV group). Fourteen patients received no preoperative therapy (control CNV group). The CNVM were stained for cytokeratin 18, CD68, CD45, intercellular adhesion molecule (ICAM)–1, E-selectin, Ki-67, Thy-1, and endostatin.

Results: No significant difference was detected in ICAM-1 and E-selectin expression between groups. The density of leukocytes in the bevacizumab CNV group (median, 271.61 cells/mm²) was higher than in the control CNV group (median, 116.87 cells/mm²; P = .07), but without significance. Density of macrophages (median, 4661.95 cells/mm²), proliferative activity (median, 160.19 cells/mm²), and percentage of Thy-1–expressing vessels (median, 100%) were significantly higher in the bevacizumab CNV group than in the control CNV group (median, 882.66 cells/mm², P < .001; median, 34.34 cells/mm², P < .001; and median, 80%, P < .001, respectively). Endostatin immunoreactivity was considerably stronger in the retina pigment epithelium (RPE)–Bruch membrane complex (median, 3; range, 2-3; P < .001), and stroma (median, 3; range, 1-3; P < .001) of the bevacizumab CNV group than control CNV group (median, 1.5; range, 0-3 and median, 1, range, 0-3, respectively).

Conclusions: Unexpectedly, CNVM from patients treated by bevacizumab are characterized by significantly high inflammatory and proliferative activity and enhanced endostatin expression. These characteristics need to be considered when protocols for combination therapies are established.

Arch Ophthalmol. 2008;126(6):782-790
investigation included Thy-1, owing to its characteristics as a cell surface marker expressed on vascular endothelial cells (EC) that are upregulated by the inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α. Endostatin is an endogenous angiogenesis inhibitor that is activated by proteases such as those derived from inflammatory cells. The antigen Ki-67 was used as a nuclear marker of cell proliferation.

METHODS

PATIENTS AND TREATMENTS

We retrospectively reviewed 38 eyes of 38 consecutive AMD patients in different centers who were surgically treated with full macular translocation surgery with CNVM extraction. All patients had clinically active disease and progression. Fourteen CNV patients without any kind of therapy prior to surgery composed the control CNV group. The clinical characteristics of these 14 patients are summarized in Table 1. Twenty-four patients received surgery following intravitreal bevacizumab injection (bevacizumab CNV group). Therapy options, including observation, conventional thermal laser photocoagulation, verteporfin PDT, intravitreal injection of triamcinolone acetone or anti-VEGF agents, and full macular translocation with 360° retinotomy and CNVM extraction were discussed with the patients. Although submacular removal of CNV was not favored by the randomized submacular surgery trials, submacular surgery trial Group B revealed that submacular surgery for predominantly hemorrhagic CNV did reduce the risk of severe visual acuity loss (loss of ≥6 Early Treatment Retinopathy Study lines) and contrast sensitivity loss (≥3 segments, using the Pelli-Robson chart at 0.5 m) significantly, in comparison to the group observed without any treatment for 24 months of follow-up. Macular translocation of the retina, a complex but potentially effective surgical method, was reported to be beneficial when CNV was related to submacular hemorrhage or RPE tear. Furthermore, improvement in distance and near visual acuity as well as in reading speed was detected at the 1-year follow-up of patients with neovascular AMD who were treated with macular translocation.

Intravitreal bevacizumab injection and surgical intervention with full macular translocation surgery were offered when (1) visual acuity was worse than 20/200 (being the minimum visual acuity to recommend the first PDT, according to the Treatment of Age-Related Macular Degeneration with Photodynamic Therapy investigation); (2) CNV was related to retina pigment epithelium (RPE) tear, chorioretinal anastomosis, or massive subretinal/intravitreal hemorrhage; (3) visual deterioration progressed and subretinal hemorrhage and RPE tear appeared following treatment; or (4) reading ability was recently lost, despite improvement in distant visual acuity after previous treatment. The clinical characteristics of the patients treated with intravitreal bevacizumab are summarized in Table 2. Reduction of intraoperative bleeding was intended with the preoperative injection of bevacizumab, as suggested in other ocular pathologies.

Each patient gave written informed consent regarding their knowledge of the off-label use and experimental nature of the treatment procedure, and the risks and benefits of all therapeutic options were discussed in detail. The study followed the guidelines of the declaration of Helsinki. Local institutional review board approval was granted for allocation and histological analysis of the specimens.

TISSUE PREPARATION AND IMMUNOHISTOLOGY

Within minutes after surgery, excised CNVM were fixed in 3.7% formalin and embedded in paraffin. After serial sections were deparaffinized and rehydrated, antigen retrieval was accomplished by proteolytic digestion with 0.5% protease XXIV (Sigma, St Louis, Missouri) for cytokeratin 18, endostatin, and Thy-1, and by heat treatment in citrate buffer in a pressure cooker under 120°C for 2 minutes for Ki-67, CD45, CD68, ICAM-1, and E-selectin.

Immunohistochemical staining with the primary mouse monoclonal antibodies specific for cytokeratin 18 (Progen, Heidelberg, Germany), ICAM-1 (Novocastra, Newcastle upon Tyne, England), E-selectin (Novocastra), CD45 (Dako, Glostrup, Denmark), and Ki-67 (Clone Ki-55; Dako) was performed using the avidin-biotin complex horseradish peroxidase method, as previously described. For E-selectin, cytokeratin 18, and ICAM-1 staining, the brown chromogen 3-diaminobenzidine was replaced with 3-amino-9-ethylcarbazole, a highly sensitive substrate chromogen (Code K3461; Dako).

Immunohistochemical staining for Thy-1, CD68, and endostatin was performed by the alkaline-phosphatase method, according to the manufacturer’s instructions (ChemMate Detection Kit, Alkaline Phosphatase/RED, Rabbit/Mouse, K3005; Dako), as previously described, using primary antihuman antibodies specific for Thy-1 (mouse anti-CD90 antibody; BD Biosciences Pharmingen, San Jose, California), CD68 (mouse, monoclonal; Dako), and endostatin (rabbit, polyclonal; Dianova GmbH, Hamburg, Germany). Color was developed using chromogen red (ChemMate Detection Kit; Dako). Hematoxylin (Chemmate, Code S2020; Dako) was used for counterstaining.

For negative controls, primary antibodies were substituted by the appropriate normal sera or omitted.

ANALYSIS

Serial sections from a specimen were analyzed by light microscopy. Immunoreactivity for ICAM-1, E-selectin, CD68, and endostatin was analyzed semiquantitatively and separately in the RPE, EC (in the perivascular area for CD68), and stroma. A grading scheme indicating the degree of labeling was used: 1, weak; 2, moderate; 3, strong. Three of 2, 1, and 0 were assigned for intense (70%-100% positive cells), moderate (40%-69% positive cells), weak (1%-39% positive cells), and absent of staining, respectively.

Each specimen was documented under ×50 magnification with a Zeiss microscope (Axioskop; Carl Zeiss, Inc, Oberkothen, Germany) connected to a digital camera (Fujix HC-300Z; Fujifilm, Tokyo, Japan). The area of each specimen was measured using the appropriate hardware and software (AxioVision, Version 3.1; Carl Zeiss, Inc, Gottingen, Germany).
All Ki-67–positive nuclei, macrophages, and leukocytes were counted in each specimen. Proliferative activity, density of leukocytes, and macrophage infiltration in each specimen were determined quantitatively by calculating the ratio of the total number of Ki-67 positive nuclei, macrophages, and leukocytes in the CNVM to the total area of the membrane.

Thy-1 expression was determined by the percentage of Thy-1–expressing vessels in the overall vascularization of each membrane.

Intensity of CD68, ICAM-1, E-selectin, and endostatin immunostaining, density of leukocyte and macrophage infiltration, proliferative activity, and Thy-1 expression in the described CNV groups were compared using the Mann-Whitney U test. Immunohistologic findings in the bevacizumab CNV group were analyzed based on timing or number of bevacizumab injections, using the Spearmann correlation test. A P value less than .05 was considered significant.

## RESULTS

Immunohistological findings are summarized in Figure 1.

### HISTOPATHOLOGICAL FINDINGS IN THE CONTROL CNV GROUP

Retina pigment epithelial cells immunoreactive for cytokeratin 18 were found in all specimens.

All but 1 membrane was vascularized. Immunohistologic stains of the specimen from a patient with massive subretinal hemorrhage (Figure 2A) are shown in Figure 2.

Expression of E-selectin was found in the RPE of all CNVM, and intensely in 4 (28.6%) membranes. E-selectin was detected in the EC of 8 CNVM (57.1%) and in the stroma of 9 CNVM (64.3%) (Figures 1A and 2B).

Expression of ICAM-1 was prominent in the RPE; it was intense in 13 CNVM (92.9%). No ICAM-1 was present in the EC of 9 CNVM (64.3%). Intense ICAM-1 expression was detected in the EC of 3 (21.4%) and in the stromal cells of 2 CNVM (14.3%) (Figures 1B and 2C).

Leukocytes detected by CD45 immunostaining were found to be present in all but 1 CNVM. The number of leukocytes in a 1-mm² area of CNVM varied between 0 and 779.6 (median, 106.64 cells/mm²) (Figure 1C). The specimen in Figure 2D has the fourth highest density of leukocyte infiltration (157.39 cells/mm²) in the control CNV group.

The density of macrophages ranged between 183.94 cells/mm² and 2826.73 cells/mm² (median, 882.66 cells/mm²) (Figure 1C). Macrophage infiltration was seen in the RPE cell layer, the perivascular area, and the stroma in 85.7% (n=14), 61.5% (n=13), and 92.8% (n=14) of the specimens. Intense macrophage infiltration was detected in the RPE cell layer of 6 (42.8%), the perivascular area of 2 (14.3%), and the stroma of 4 (28.6%) CNVM (Figures 1D and 2E).

Vessels expressing Thy-1 were detected in 13 of 14 specimens (92.8%), in varying percentages of vessels (median, 80.0%; range, 0%-100%). In 4 (28.6%) CNVM, all vessels displayed Thy-1 (Figure 2F).
Immunoreactivity to endostatin was detected in the RPE–Bruch membrane complex in 10 (71.4%) membranes. Endostatin was immunonegative in vessels in only 7.7% (n=1) of the specimens. Within the stroma, endostatin was expressed by fibroblast-like and inflammatory cells in 11 (78.6%) specimens (Figures 1E and 2G).

Proliferative activity varied between 6.67 and 514.08 Ki-67–expressing nuclei/mm² (median, 34.34 nuclei/mm²) (Figure 1C). Proliferating cells belonged mostly to inflammatory infiltrate within the stroma (Figure 2H).

In 12 (85.7%) CNVM, proliferative activity was less than 160 Ki-67–expressing nuclei/mm². The specimen in Figure 2H has the second highest proliferative activity among the control CNV group (511.05 nuclei/mm²).

**HISTOPATHOLOGICAL FINDINGS IN THE BEVACIZUMAB CNV GROUP**

In the bevacizumab CNV group (Figure 3A), the RPE had E-selectin in 18 (75.0%) CNVM. The EC were E-selectin immunonegative in 11 (45.8%) CNVM. Intense E-selectin staining was detected in the RPE of 6 (25%) and the EC of 8 (33.3%) CNVM. Stromal cells expressed E-selectin in 12 (50.0%) CNVM (Figures 1A and 3B). Expression of E-selectin in the bevacizumab CNV group was comparable to the control CNV group.

Immunoreactivity for ICAM-1 was detected in the RPE of 21 CNVM (87.5%) and intensely in 18 CNVM (75%). Intense ICAM-1 expression was found in the EC of 5 (20.8%) and stromal cells of 1 (4.2%) CNVM (Figures 1B and 3C). Expression of ICAM-1 was not significantly different from the control CNV group.

Leukocytes were found in all specimens (median, 271.61 cells/mm²; range, 33.50-2351.01 cells/mm²) (Figure 3D). The density of leukocytes seemed higher than in the control group, but without statistical significance (Figure 1C; P=.07).

Macrophages were present in all CNVM (median, 4661.95 cells/mm²; range, 321.01-61821.16 cells/mm²) (Figures 1C and 3E). The density of macrophages was significantly higher than that of the control CNV group (P<.001). Moderate to strong macrophage infiltration was detected within the RPE cell layer and in the perivascular area in 18 (75%) specimens and in the stroma in 17 (70.8%).

Figure 1. Graphs showing E-selectin (A) and intercellular adhesion molecule (ICAM)-1 (B) immunostaining intensity, density of leukocytes and macrophages (CD68) and proliferative activity (C), CD68 immunoreactivity (D), and intensity of endostatin staining (E) in choroidal neovascular membranes (CNVM) without any kind of previous therapy (control choroidal neovascularization [CNV] group) and CNVM excised after intravitreal injection of bevacizumab (Avastin; Genentech, Inc, South San Francisco, California) (bevacizumab CNV group). Expression of ICAM-1, E-selectin, CD68, and endostatin immunostaining in retina pigment epithelium (RPE), endothelial cells (EC) (perivascular area for CD68), and stroma were evaluated separately and semiquantitatively as intense (70%-100% positive cells), moderate (40%-69% positive cells), mild (1%-39% positive cells), or absent (A,B,D,E). All Ki-67–positive nuclei, CD45-immunoreactive leukocytes, and CD68-immunopositive macrophages were counted in each specimen. Proliferative activity and density of leukocytes and macrophages were determined separately for each CNVM by the ratio of total number of proliferating cells, leukocytes, and macrophages to the total area of the membrane (mm²); their median values were shown in (C). ©2008 American Medical Association. All rights reserved.
Immunoreactivity of CD68 was significantly more intense in the bevacizumab CNV group in the perivascular area (median, 3) and stroma (median, 3) than in the control CNV group (median, 1; \( P = .001 \) and median, 2; \( P = .03 \), respectively).

In the CNVM of all patients treated with bevacizumab, all vessels were lined with EC displaying Thy-1 (median, 100.0%) (Figure 3F). The percentage of Thy-1–expressing vessels was significantly higher in the bevacizumab CNV group (\( P < .001 \)).

The RPE–Bruch membrane complex displayed endostatin in all but 1 CNVM either strongly (70.8%; \( n = 17 \)) or moderately (25.0%; \( n = 6 \)). Endostatin was intensely expressed in the vessels of 20 CNVM (87.3%). Stromal cells displayed endostatin in all CNVM and strongly in 21 (87.5%) membranes (Figures 1E and 3G). Endostatin was significantly stronger in the RPE–Bruch membrane complex (median, 3; range, 2-3; \( P < .001 \)) and stroma (median, 3; range, 1-3; \( P < .001 \)) of the bevacizumab CNV group than the control CNV group (median, 1.5; range, 0-3 and median, 1; range, 0-3, respectively).

Proliferative activity (median, 204.33 cells/mm\(^2\); range, 3.61-1886.135 cells/mm\(^2\)) in the bevacizumab CNV group was significantly higher than that in the control CNV group (\( P = .007 \)) (Figures 1C and 3H).
Immunohistologic findings in the bevacizumab CNV group did not show major differences based on either timing or number of bevacizumab injections (Table 3). Based on previous application of PDT, only E-selectin expression showed a major difference, being significantly stronger in the EC of patients in the bevacizumab CNV group treated with PDT previously (n = 4) than those in the bevacizumab CNV group without any previous PDT (n = 20) (Table 3).

Table 3. Evaluation of Histopathological Findings in Bevacizumab CNV Group With Regard to Application of Bevacizumab and PDTa

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<td>Proliferative activity</td>
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Abbreviations: CNV, choroidal neovascularization; CNVM, choroidal neovascular membrane; EC, endothelial cells; ICAM, intercellular adhesion molecule; PDT, photodynamic therapy; RPE, retina pigment epithelium.

aIntensity of E-selectin, ICAM-1, and endostatin immunostainings in RPE, EC, and stroma. Density of leukocyte and macrophage infiltration, proliferative activity, and Thy-1 expression in bevacizumab CNVM were analyzed based on timing or number of bevacizumab injections with the Spearman correlation test and compared based on previous application of verteporfin PDT using the Mann-Whitney U test. P < .05 was considered significant.

Vascular endothelial growth factor is expressed in the chorioid of human eyes, and polarized secretion of VEGF in the RPE is vital for choriocapillaris development and maintenance. In AMD, various factors such as free radicals or complement components may upregulate VEGF expression. Vascular endothelial growth factor, chemotactic for inflammatory cells, activates monocytes and macrophages and induces expression of ICAM-1 and E-selectin on vascular EC to facilitate adhesion and migration of inflammatory cells. Macrophages synthesize the cytokines IL-1 and TNF-α, which enhance ICAM-1 expression in EC and in the RPE, and induce inflammatory cell infiltration further. There is a closed but amplifying circuit between VEGF and inflammatory cells, because leukocytes and macrophages can produce VEGF themselves and also induce VEGF production in the RPE through IL-1 and TNF-α in human CNVM. Vascular endothelial growth factor, in turn, stimulates growth and maintenance of the CNVM. Because VEGF and inflammation seem to closely interact with each other, VEGF inhibition by bevacizumab might influence, or possibly inhibit, the inflammatory activity in CNV.

In the specimens treated with bevacizumab, expression of ICAM-1 and E-selectin was comparable to that of the control CNV cases without previous therapy. However, density of leukocytes, and especially of macrophages, was higher in the bevacizumab CNV group than in the control CNV group. Consequently, the percentage of Thy-1—expressing vessels, a mirror of TNF-α and IL-1 activity, was also significantly higher. Macrophages, reported to be present in 60% of the surgically excised CNVM, seem to play a causative role in neovascular AMD pathogenesis. In aged RPE cells, accumulation of oxidation products seems to induce angiogenic and chemotactic factors like monocyte chemoattractant protein 1. In healthy human eyes, recruited macrophages may have a physiological role in removing the accumulated subretinal debris. However, excess oxidized lipids in the eyes of patients with AMD were suggested to upregulate the release by macrophages of proteolytic enzymes that damage the Bruch membrane and contribute to CNV development. Macrophages, in addition to stimulating VEGF, can induce EC proliferation and migration through the release of cytokines, proteases, and growth factors. Therefore, experimental studies for potential new antiangiogenic treatment strategies target the complement system, ICAM, macrophages, monocytes, and TNF. These were shown to inhibit leukocyte infiltration and VEGF expression and, therefore, inhibit CNV development or progression in experimental studies. In experimental models, early intravitreal injection of VEGF-A antibody after CNV induction was shown to inhibit CNV and choroidal macrophage in-
filtration through suppression of monocyte chemoattractant protein 1 expression in the RPE-choroid complex.\textsuperscript{38} Hence, anti-VEGF drugs might be expected to have an anti-inflammatory effect.\textsuperscript{39}

Our results were truly unexpected. Inhibition of VEGF with bevacizumab was related to a prominent inflammatory reaction, instead of an anti-inflammatory effect, in comparison to the control CNV group. This might be for several reasons.

First, the Fc portion of bevacizumab in CNV may stimulate a cellular immune response through Fc receptors in immune cells. Bevacizumab was shown to penetrate the retina in an experimental study.\textsuperscript{40} Anatomical defects in the Bruch membrane created by proteolytic enzymes and CNV itself might facilitate the passage of bevacizumab molecules through the RPE to the CNVM in the eyes of patients with AMD.

Second, VEGF acts as a survival factor for EC by inhibiting apoptosis.\textsuperscript{37,61} Deprivation and/or inhibition of VEGF was shown to induce vascular regression through EC apoptosis, especially in neovascular vessels.\textsuperscript{51-65} During vascular regression, macrophages regulate EC and pericyte apoptosis in capillaries. Leukocytes release human neutrophil peptides that are proapoptotic for EC.\textsuperscript{66} Dostatin was decreased in the RPE and choroid of bevacizumab-treated CNV.\textsuperscript{19} Furthermore, endostatin was shown to inhibit CNV.\textsuperscript{70} Previously, macrophage depletion with liposomal clodronate was shown to inhibit CNV development.\textsuperscript{39} In another experimental study, macrophage depletion results in EC survival and persistence of functional capillaries.\textsuperscript{48,49} Therefore, increased inflammatory cell infiltration following bevacizumab injection may reflect part of the vascular regression process in CNV, based on debris removal by macrophages.

Third, macrophages might be involved in CNV inhibition. In mice deficient in monocyte chemoattractant protein 1 or its receptor, accumulating complement factors or IgG stimulated VEGF expression in the RPE and, therefore, CNV development.\textsuperscript{39} In another experimental study, inhibition of macrophage entry into the eye stimulated CNV, whereas direct injection of macrophages inhibited CNV.\textsuperscript{70} Previously, macrophage depletion with liposomal clodronate was shown to inhibit CNV development.\textsuperscript{48,49} However, reduction of neovascularization in these studies was recently suggested to be due to the direct toxicity of liposomes on EC rather than macrophage depletion itself.\textsuperscript{70}

Last, inflammatory cells are known to contribute to antiangiogenesis through activation of endogenous angiogenesis inhibitors such as endostatin. Macrophages and leukocytes release proteases such as matrix metalloproteases.\textsuperscript{20} Proteases cleave endostatin that is bound to collagen XVIII in vascular basement membranes and the Bruch membrane. Through this cleavage, endostatin is released and activated.\textsuperscript{21} In our series, endostatin was significantly more intense in the RPE and stroma of the inflammatory active bevacizumab CNV group than in the control CNV group. This has important consequences because endogenous endostatin was shown to inhibit CNV.\textsuperscript{19} Furthermore, endostatin was decreased in the RPE and choroid of human eyes with AMD, and its deficiency was suggested to predispose CNV formation.\textsuperscript{21}

Vascular endothelial growth factor was shown to be an important mitogenic factor for choroidal EC\textsuperscript{37,57,72,73} and also for RPE cells.\textsuperscript{74} However, whether anti-VEGF toxins or antibodies inhibit proliferation of human choroidal and RPE cells is controversial in in vitro settings.\textsuperscript{75-77} Recently, we have shown the antiproliferative effect of bevacizumab on EC in in vitro studies.\textsuperscript{78,79} Still, in vitro results may not reflect the ongoing process in vivo. In the bevacizumab CNV group, proliferative activity was significantly higher than in the control CNV group. Proliferating cells were located within the stroma and originated from the inflammatory infiltrate rather than the RPE or EC. In our previous work, proliferative activity was correlated with inflammatory activity, and was significantly higher in inflammatory active specimens than in inflammatory inactive ones.\textsuperscript{34,80}

In our series, timing of the bevacizumab injection prior to surgery varied between 1 and 154 days. Four of the 24 patients were treated with 2 to 5 bevacizumab injections preoperatively. However, immunohistologic findings in the bevacizumab CNV group did not show major differences based on timing or number of injections. Four of the patients were treated with PDT 39 to 343 days before bevacizumab injection. Of the antigens investigated, only E-selectin was found to be significantly more intense in the EC of the bevacizumab CNV patients treated previously with PDT. However, our previous studies\textsuperscript{34} have shown that immunohistopathological findings dramatically change early (3 days) after PDT when compared with either longer intervals following PDT or the control CNV group. The implications of PDT-bevacizumab combination therapy on inflammation and angiogenesis, therefore, need to be evaluated in a higher number of samples excised early after combination therapy.

To our knowledge, this is the first clinicopathological study evaluating inflammation and proliferation in human CNV treated with an anti-VEGF agent. To interpret the results properly, the following limitations need to be taken into account: first, histopathological specimens reflect only a time window within a dynamic process. Therefore, we selected homogenous control and treatment groups that were comparable in their clinics. Second, in contrast to experimental studies, time between treatment and tissue acquisition varies. Nevertheless, data retained by histopathological examination of human samples may be closer to the truth than any data collected by in vitro or in vivo experiments.\textsuperscript{81}

The results of this study, revealing high inflammatory and proliferative activity, were truly unexpected and appear to be, to some extent, paradoxical to the inhibition of angiogenesis. The question is how to interpret this data in view of promising clinical results related to a decrease in disease activity following intravitreal injection of bevacizumab. There is growing knowledge that inflammatory reactions exert not only proangiogenic activities, but are also involved in vascular regression and angiogenesis inhibition. We assume that a similar process may be reflected in our histopathological results. Our results reveal enhanced expression of the endogenous angiogenesis inhibitor endostatin in these inflammatory active bevacizumab CNVM. Still, our understanding of treatment modalities and combination therapies needs to be further evaluated to implement the optimal treatment
strategies and protocols for neovascular AMD. High inflammatory and proliferative activity as well as enhanced endostatin expression in bevacizumab-treated CNV should be considered when combination therapies with bevacizumab are planned.11,82,83

Submitted for Publication: July 19, 2007; final revision received December 17, 2007; accepted December 22, 2007.

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Author Contributions: Olcay Tatar and Salvatore Grisanti had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding Support: This work was supported by grants from the Vision 100 Foundation and the Jung Foundation.

Financial Disclosure: None reported.

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